Hepatic carbon flux after re-feeding in the glycogen-storage-disease (*gsd/gsd*) rat

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In this study we utilized the phosphorylase *b* kinase-deficient (*gsd/gsd*) rat as a model of hepatic substrate utilization where there is a constraint on glycogenesis imposed by the maintenance of high glycogen concentrations. Glucose re-feeding of 48 h-starved *gsd/gsd* rats led to suppression of hepatic glucose output. In contrast with the situation in normal rats, activation of the pyruvate dehydrogenase complex and lipogenesis was observed. It is suggested that impeding glycogenic flux may divert substrate into lipogenesis, possibly via activation of the pyruvate dehydrogenase complex.

INTRODUCTION

Glucose is not efficiently utilized by the liver for the synthesis of glycogen or fatty acids (reviewed by Katz *et al.*, 1986). Instead, the primary role of glucose is that of a regulator, stimulating either glycogen synthesis or lipogenesis, depending on the physiological state (Hems *et al.*, 1972; Clark *et al.*, 1974; Boyd *et al.*, 1981). Lactate and related C₃ metabolites of glucose serve as precursors for both glycogenesis and lipogenesis (Hems *et al.*, 1972, 1975; Brunengraber *et al.*, 1973; Salmon *et al.*, 1974), but these processes do not necessarily occur concomitantly (see Postle & Bloxham, 1980). For example, there is now convincing evidence that, after re-feeding, hepatic gluconeogenesis from lactate continues, with the redirection of gluconeogenic product (glucose 6-phosphate) from glucose release towards glycogen synthesis (Shikama & Ui, 1978; Sugden *et al.*, 1983; Newgard *et al.*, 1983, 1984), whereas pyruvate dehydrogenase (PDH) activity and lipogenic rate remain low (Holness *et al.*, 1986). It has been proposed that it is only after the replenishment of hepatic glycogen stores that glucose enters glycolysis and may be utilized via PDH for fatty acid synthesis (Van Schaftingen *et al.*, 1980a,b; Hue *et al.*, 1984; Kuwajima *et al.*, 1984). However, previous studies have demonstrated that acute suppression of glycogenic flux by 3-mercaptopicolinate (MPA) leads to activation of hepatic lipogenesis (Sugden *et al.*, 1983; Newgard *et al.*, 1984), implying that a mechanism may exist to divert substrate into lipogenesis in the event that glycogenesis is impeded.

The glycogen-storage-disorder (*gsd/gsd*) rat lacks hepatic phosphorylase *b* kinase. Glycogen synthase is also relatively inactive, probably because of inhibition of synthase phosphatase by high glycogen concentrations (Watts & Malthus, 1980). As with normal (i.e. *GSD/GSD*) rats, hepatic gluconeogenesis contributes to the maintenance of glycaemia in the starved state (Conaglen *et al.*, 1985), but liver glycogen concentrations remain high (Clark *et al.*, 1980; Malthus *et al.*, 1980). In the present experiments we have compared the responses of 48 h-starved normal or *gsd/gsd* rats to the provision of glucose. The nature of the lesion in the *gsd/gsd* rats allows the important question of the relationship between

glycogenesis and lipogenesis as alternative fates of lactate in the liver after re-feeding to be addressed without pharmacological intervention. The specific objectives of the work were (i) to determine whether the impaired ability to synthesize glycogen from lactate is associated with its diversion into lipogenesis, and (ii) to examine whether activation of lipogenesis is paralleled by re-activation of hepatic PDH.

MATERIALS AND METHODS

Sources of materials were as in Sugden *et al.* (1983).

Manipulations *in vivo*

Female albino Wistar rats (*GSD/GSD*) or female *gsd/gsd* rats, a substrain of the NZR/Gd line, were fed *ad libitum* or starved for 48 h (in grid-bottomed cages).

Experiments were started between 08:30 and 09:30 h, when glucose (2 mmol/100 g body wt.) or water (1 ml/100 g body wt.) was administered intragastrically (see Sugden *et al.*, 1983). As glucose tolerance is unaffected by phosphorylase *b* kinase deficiency (Conaglen *et al.*, 1985), blood and liver samples were obtained at a single time point (1 h after the intragastric load). ³H₂O was administered by intraperitoneal injection at the time of the glucose load.

Lipogenesis and glycogen synthesis

Rates of lipogenesis were estimated as ³H incorporation from ³H₂O into tissue saponifiable fatty acid (Stansbie *et al.*, 1976). Rates of glycogen synthesis were estimated (in the same tissue samples) as the incorporation of ³H from ³H₂O into glycogen (Postle & Bloxham, 1980).

The incorporation of ³H from ³H₂O into glycogen occurs at the hexose phosphate level and is dependent on the specific radioactivity of the cytosolic ³H₂O pool (see Postle & Bloxham, 1980). There is no intracellular compartmentation of ³H₂O (Postle & Bloxham, 1980). Thus, although the *gsd/gsd* livers contain high concentrations of glycogen (with associated structurally bound

Abbreviations used: PDH, pyruvate dehydrogenase; MPA, 3-mercaptopicolinate; NEFA, non-esterified fatty acids.

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Table 1. Blood ketone-body (acetoacetate and 3-hydroxybutyrate) and NEFA concentrations in control (*GSD/GSD*) or phosphorylase *b* kinase-deficient (*gsd/gsd*) 48 h-starved rats after the administration of water or glucose

For experimental details see the text. Results are means $\pm$ S.E.M. for four to eight rats. Statistically significant effects of phosphorylase *b* kinase deficiency are indicated by ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001, and those of glucose administration by ^d*P* < 0.01, ^e*P* < 0.001.

Sampling site	Treatment	Rats ...	Metabolite concn. (mM)			
			NEFA		Ketone bodies	
			<i>GSD/GSD</i>	<i>gsd/gsd</i>	<i>GSD/GSD</i>	<i>gsd/gsd</i>
Hepatic portal vein	Water		0.80 $\pm$ 0.05	0.99 $\pm$ 0.05 ^a	2.04 $\pm$ 0.07	1.50 $\pm$ 0.09 ^c
	Glucose		0.40 $\pm$ 0.05 ^e	0.51 $\pm$ 0.14 ^d	0.34 $\pm$ 0.05 ^e	0.23 $\pm$ 0.04 ^e
Aorta	Water		0.74 $\pm$ 0.05	0.69 $\pm$ 0.06	2.24 $\pm$ 0.12	1.58 $\pm$ 0.12 ^b
	Glucose		0.25 $\pm$ 0.03 ^e	0.41 $\pm$ 0.03 ^{bd}	0.37 $\pm$ 0.06 ^e	0.25 $\pm$ 0.06 ^e
Hepatic vein	Water		0.74 $\pm$ 0.05	0.85 $\pm$ 0.04	2.40 $\pm$ 0.10	1.59 $\pm$ 0.11 ^c
	Glucose		0.44 $\pm$ 0.06 ^d	0.44 $\pm$ 0.09 ^d	0.36 $\pm$ 0.06 ^e	0.26 $\pm$ 0.05 ^e

Table 2. Hepatic glycogen concentrations and synthesis rates in control (*GSD/GSD*) or phosphorylase *b* kinase-deficient (*gsd/gsd*) 48 h-starved rats after water or glucose administration

For experimental details see the text. Results are means $\pm$ S.E.M. for five to eight rats. Statistically significant effects of phosphorylase *b* kinase deficiency are indicated by ^a*P* < 0.001, and those of glucose administration by ^b*P* < 0.05, ^c*P* < 0.001.

Rats	Treatment	Glycogen concn. (μ mol of glucose/g wet wt.)	Glycogen synthesis rate (μ g-atoms of H/h per g wet wt.)
<i>GSD/GSD</i>	Water	7 $\pm$ 1	33.7 $\pm$ 9.2
	Glucose	46 $\pm$ 9 ^c	133.8 $\pm$ 13.0 ^c
<i>gsd/gsd</i>	Water	691 $\pm$ 35 ^a	29.6 $\pm$ 2.5
	Glucose	730 $\pm$ 22 ^a	37.5 $\pm$ 1.9 ^{ab}

water), there is no reason *a priori* to believe that rates of glycogen synthesis will be underestimated in these rats. Postle & Bloxham (1980) have demonstrated that measurement of glycogen synthesis rates by the incorporation of ³H from ³H₂O is reliable at the high glycogen concentrations found in meal-fed rats (Postle & Bloxham, 1980). Structurally bound ³H₂ was removed by the following procedure. Tissue (< 200 mg) was extracted (5 min, 100 °C) in 0.3 ml of 30% (w/v) KOH, and the glycogen was precipitated (overnight, 2 °C) with 1 ml of ethanol containing a drop of satd. Na₂SO₄. The precipitate was isolated by centrifugation and washed with ethanol (twice) and acetone (once). After drying at 90 °C, the glycogen was digested with amyloglucosidase in 20 mM-sodium acetate buffer, pH 4.8, and freeze-dried.

Enzyme assays

Pyruvate dehydrogenase complex (active form, PDH_a) and citrate synthase activities were measured in freeze-clamped liver extracts as described by Caterson *et al.* (1982). PDH_a activities are expressed relative to citrate synthase, to correct for possible differences in mitochondrial content/extraction (see, e.g., Caterson *et al.*, 1982; Holness *et al.*, 1986; Holness & Sugden, 1987). A unit of enzyme converts 1 μ mol of substrate into product/min at 30 °C.

Metabolite assays

Glucose and ketone-body (3-hydroxybutyrate plus acetoacetate) concentrations were routinely determined by KOH-neutralized HClO₄ extracts of blood sampled from the hepatic portal vein, the hepatic vein and the aorta. NEFA concentrations were measured in plasma. Lactate, pyruvate, citrate and glycogen concentrations were determined in extracts of freeze-clamped livers [see Bergmeyer (1974) for details of methods].

Statistics

The results are presented as means $\pm$ S.E.M. for 4–13 rats. Statistical differences were assessed by Student's unpaired *t* test.

RESULTS AND DISCUSSION

Fatty acid and ketone-body concentrations after re-feeding

Starved normal and starved *gsd/gsd* rats had similar concentrations of NEFA, but the concentrations of ketone bodies were lower (by approx. 30%) in *gsd/gsd* rats (Table 1). Both groups of rats responded to the provision of glucose with a decline in the concentrations of lipid fuels (Table 1), but, again, ketonaemia was lower in the *gsd/gsd* rats. These findings may be taken to

Fig. 1. Portal minus hepatovenous concentration differences after re-feeding

Phosphorylase *b* kinase-deficient (*gsd/gsd*; ●, ▲) or control (*GSD/GSD*; ○, □) rats were given glucose (●, ○) or water (▲, △) by intragastric intubation under ether anaesthesia. Blood was sampled after 1 h while the rats were anaesthetized with sodium pentobarbitol (6 mg/100 g body wt., 5 min). Portal minus hepatovenous glucose-concentration differences $[P - V]$ are plotted as a function of the portal glucose concentration $[P]$ for each rat.

indicate that a greater proportion of fatty acyl-CoA is esterified rather than oxidized.

Hepatic glycogen synthesis and glucose handling in starvation and after re-feeding

Glycogen concentrations in starved (i.e. water-fed) *GSD/GSD* rats were, as expected, low (Table 2). High hepatic glycogen concentrations were maintained after starvation in *gsd/gsd* rats, as has been noted previously (Clark *et al.*, 1980; Malthus *et al.*, 1980). The significant incorporation of ^3H from $^3\text{H}_2\text{O}$ into glycogen in the starved state, despite the removal of structurally bound water (see the Materials and methods section), supports the suggestion of Mvumbi & Stalmans (1987) that hepatic glycogen is continually being synthesized and degraded, without net deposition, in the starved state.

However, although the presence of high concentrations of glycogen may minimize the occurrence of such a cycle (Hutson *et al.*, 1976; see Mvumbi & Stalmans, 1987), the incorporation of ^3H into glycogen of *gsd/gsd* rats indicates that additional control mechanisms may operate, or that ^3H is incorporated from $[^3\text{H}]$ hexose phosphate via reversal of the phosphorylase reaction.

Glucose administration to 48 h-starved *GSD/GSD* rats increased hepatic glycogen content. There was an approx. 4-fold increase in the rate of glycogen synthesis, estimated as the incorporation of ^3H into glycogen (Table 2). The ratio of ^3H incorporated to glucosyl-unit synthesized (2.6:1) approximates to that observed by Postle & Bloxham (1980) in meal-fed rats. Increases in glycogen deposition in *gsd/gsd* rats were not statistically significant (Table 2). The rate of ^3H incorporation into glycogen was increased in response to glucose by only 27%.

Although affected *gsd/gsd* rats exhibited somewhat lower blood glucose concentrations in the starved state, marked hypoglycaemia was not evident (see Fig. 1). The rate of hepatic gluconeogenesis *in vivo* (estimated by the incorporation of $[^{14}\text{C}]$ bicarbonate into $[^{14}\text{C}]$ glucose) is unchanged in *gsd/gsd* rats (Conaglen *et al.*, 1985) and net hepatic glucose output can then be inferred from the negative portal-minus-hepatovenous glucose-concentration difference ($[P - V]$) observed in the present study (Fig. 1). A positive value of $[P - V]$ indicated net hepatic glucose uptake over glucose administration in both *GSD/GSD* and *gsd/gsd* rats, with a linear relationship between the glucose uptake and the portal glucose concentration in each case (Fig. 1). As net glycogen deposition is not significantly increased on re-feeding in *gsd/gsd* rats, it may be concluded that the effects of glucose re-feeding to suppress glucose output are not necessarily dependent on the diversion of gluconeogenic product towards glycogen synthesis. The results therefore provide indirect support for the concept proposed by Newgard *et al.* (1984) that glucose may suppress hepatic glucose output directly via inhibition of glucose-6-phosphatase.

Substrate utilization via the PDH complex

Hepatic PDH activities and lipogenic rates were low in the starved state in both *GSD/GSD* and *gsd/gsd* rats (Table 3). As previously noted by Holness *et al.* (1986), PDH activity was refractory to re-activation in response

Table 3. Hepatic PDH complex activity, lipogenic rate, lactate plus pyruvate and citrate concentrations in control (*GSD/GSD*) and phosphorylase *b* kinase-deficient (*gsd/gsd*) 48 h-starved rats after water or glucose administration

For experimental details see the text. Results are means $\pm$ S.E.M. for 5–11 rats. Statistically significant effects of phosphorylase *b* kinase deficiency are indicated by $^aP < 0.01$, $^bP < 0.01$, $^cP < 0.001$, and those of glucose administration by $^dP < 0.05$, $^eP < 0.01$, $^fP < 0.001$.

Rats	Treatment	PDH complex activity (munits/unit of citrate synthase)	Lipogenesis (μg -atoms of H/h per g wet wt.)	Hepatic metabolite concn. ($\mu\text{mol/g}$)	
				Lactate plus pyruvate	Citrate
<i>GSD/GSD</i>	Water	9.3 ± 0.6	4.2 ± 1.1	0.76 ± 0.05	0.21 ± 0.01
	Glucose	9.4 ± 0.8	4.2 ± 0.2	1.35 ± 0.14^e	0.34 ± 0.03^e
<i>gsd/gsd</i>	Water	8.7 ± 1.4	5.6 ± 0.7	0.35 ± 0.02^c	0.17 ± 0.02
	Glucose	22.5 ± 4.4^{ad}	14.9 ± 2.0^{ef}	1.35 ± 0.16^f	0.21 ± 0.03^b

to re-feeding after starvation in *GSD/GSD* rats, but a novel finding was that hepatic PDH activities and lipogenic rates were increased in parallel in *gsd/gsd* rats.

In the fed state, where PDH is active, citrate is a major lipogenic precursor (Brunengraber *et al.*, 1978). In the starved state the origin of the acetyl-CoA used for hepatic lipogenesis has not been positively identified, but use of an inhibitor of ATP citrate lyase has excluded citrate as precursor (Triscari & Sullivan, 1977; Brunengraber *et al.*, 1978; Sugden *et al.*, 1982). Increases in lipogenesis in glucose-re-fed MPA-treated rats (Sugden *et al.*, 1983) are not associated with utilization of citrate as lipogenic precursor (Sugden & Palmer, 1986), and PDH remains inactive (Holness *et al.*, 1987). Under these circumstances marked increases in citrate concentrations are observed (Holness *et al.*, 1987). In contrast, increases in lipogenesis in response to re-feeding in *gsd/gsd* rats are accompanied by activation of PDH, and citrate does not accumulate (Table 3). It is attractive to suggest that the redirection of carbon flux towards lipogenesis, when glycogen concentrations are high, may be accompanied by increased use of lactate (via PDH and citrate) as lipogenic substrate.

Marchington *et al.* (1987) have detected an effect of insulin *in vitro* to reverse the effect of starvation on hepatocyte PDH activity. This effect took 4 h to complete, although it was detectable within 1 h. The response to insulin was associated with a decrease in the activities of PDH kinase and PDH-kinase activator protein. It would seem reasonable to suggest that the increase in PDH activity observed in *gsd/gsd* rats may also be a consequence of decreased activity of kinase or kinase activator. The more rapid response to re-feeding in *gsd/gsd* rats may arise because hepatic fat oxidation (indicated by the degree of ketonaemia) is diminished (Table 1).

Lipogenesis versus glycogenesis

Lipogenesis is normally refractory to re-activation after re-feeding. However, increases in hepatic lipogenesis in response to intragastric glucose may be achieved even in rats starved for 48 h under conditions where glycogen synthesis is inhibited, either pharmacologically at the level of disposal of oxaloacetate (MPA), or, as in the present experiments (via a genetic lesion) at the level of disposal of glucose 6-phosphate. In both instances, glucose-stimulated rates of lipogenesis are similar in magnitude to those observed in fed rats [see Clark *et al.* (1980) and Sugden *et al.* (1981) for details of fed rats], suggesting that lipogenic capacity does not restrict lipogenesis in the starved state. The results suggest the existence of a mechanism to divert substrate into the lipogenic pathway in the event that glycolytic flux is restricted. The delayed operation of such a diversionary mechanism may explain the failure to increase hepatic lipogenesis immediately in *GSD/GSD* rats after re-feeding, and may ensure that available substrate is sequestered as carbohydrate rather than lipid.

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